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NO. 374

MENINGOCOCCAL CONTROL IN THE CANADIAN FORCES 1. EVALUATION OF DISINFECTANTS (U)

by

L.A. White and M.R. Spence

Technical Program 16 - Operational Medicine

and

Task DPM 19

JULY 1977

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ABSTRACT

Savlon, a disinfectant composed of 15% Cetavalon and 1.5% Hibitane gluconate, was found to be an exceptionally good agent for the control of *Neisseria meningitidis*. At a dilution of 1:3000, cells were totally destroyed in 10 minutes when suspended in Neisseria Chemically Defined Medium. Savlon was greater than 30 times more effective than the standard reference, phenol. A coal tar type (20FDA) disinfectant commonly used in the Canadian Forces was less effective than phenol. Based on the results of this study, it is recommended that Savlon (1:250 in water) can be routinely used as a surface disinfectant in all areas frequented by recruits at Canadian Forces Recruit Schools, and that it replace the coal tar type disinfectant which is occasionally used for that purpose in recruit barracks at Canadian Forces Base Cornwallis. (U)

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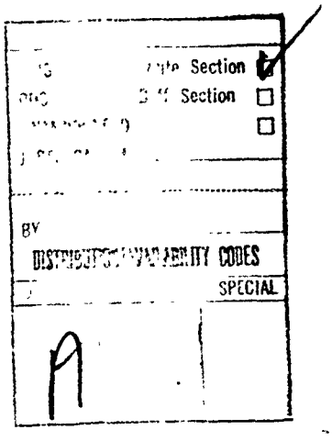

MENINGOCOCCAL CONTROL IN THE CANADIAN FORCES I. EVALUATION OF DISINFECTANTS (U)

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INTRODUCTION

The Canadian Forces have been concerned with sporadic outbreaks of meningococcal meningitidis among recruits, usually 4-fold or more in excess of normal attack rates for the similar age group in the general population. Under the auspices of the Surgeon General, a comprehensive program has been established to delineate factors involved in transmission of *Neisseria meningitidis* in recruit schools located at Canadian Forces Base (CFB) Cornwallis, Nova Scotia, and CFB St. Jean, Quebec (1). Cases of overt disease were generally accompanied by abnormally high nasopharyngeal carrier rates among other members of the same course. In the winter of 1972, carrier rates reached as high as 91% (2). A continuing survey has revealed extremely high carrier rates, generally in excess of 65% with several courses reaching 100% during the winter period. Existence of high carrier rates does not guarantee occurrence of disease cases, however.

A study conducted in May, 1973, revealed the presence of *N. meningitidis* in the air (3) at CFB Cornwallis at levels greatly in excess of those reported by Artenstein and his co-workers (4, 5). These high

numbers were apparently associated with the fact that the classes contained a high proportion of apparently healthy recruits who were carrying this organism in the nasopharynx. Highest aerosol concentrations were observed in the gymnasium, swimming pool and shower areas and in barracks immediately after wake-up. Surface samples collected in these areas revealed substantial numbers of *N. meningitidis* which had survived for periods of at least one day. Studies with artificially-generated aerosols (6) have confirmed that this organism is much more resistant to death in aerosol than has been generally assumed. This resistance to aerosol death, and the significant surface contamination observed, gave rise to the speculation that secondary aerosolization of deposited organisms could significantly contribute to the high aerosol levels, and thus increase the risk of transmission of the carrier state.

This study was initiated to determine, firstly, the *in vitro* effectiveness of Savlon (R) and a coal tar disinfectant, in common use at CFB Cornwallis, on four test strains of *N. meningitidis*, including two isolated at that base. The second part of the study was to recommend means of controlling surface contamination.

MATERIALS AND METHODS

Bacterial Strains

Four strains of *N. meningitidis* were employed in this study. Two were obtained from the Laboratory Centre for Disease Control (LCDC), Department of National Health & Welfare, Ottawa: 2241 (serogroup C) and 547 (Slaterus serogroup Y). Two additional serogroup B strains, one isolated from the air and the other from a sputum plate, were obtained at CFB Cornwallis.

Disinfectants

Standard Hospital Concentrate Savlon (15% Cetavlon and 1.5% Hibitane gluconate) (Ayerst, McKenna and Harrison Ltd., Montreal, P.Q.), coal tar type (20FDA) disinfectant (15-GP-3) and phenol U.S.P. were employed. Savlon is routinely used at CFB Cornwallis in 1:250 dilution in water, whereas coal tar type is normally diluted 1:80. Gas chromatographic

analysis of the coal tar disinfectant showed it to be composed of phenol - 48%, m, o and p-cresols - 40%, other substituted cresols - 11% and water - 1%.

Evaluation of Disinfectants

The standard U.S. Food and Drug Administration (FDA) Phenol Coefficient Method (7), and variations of it, was found to be unsuitable. The following technique was devised to determine the relative effectiveness of the disinfectants.

1. *N. meningitidis* strains were grown in Neisseria Chemically Defined Medium (NCDM) (8) at 35°C for 16 to 20 hours in an atmosphere of 5% CO_2 in air. Standardized inocula were used.

2. Stock solutions of phenol, coal tar disinfectant and Savlon, 5, 5 and 0.5 percent respectively, were prepared in NCDM.

3. Sufficient stock solution was added to test tubes to give the desired final concentration in a total of 15 ml. Sterile NCDM was then added to bring the volume to 10 ml and after temperature equilibration for 30 minutes at 35°C, 5 ml of cell suspension was added to each. Final concentrations were between 5×10^7 and 1×10^8 Colony Forming Units (CFU) per ml.

4. Tubes were incubated in a water bath at 35°C. Aliquots were removed after 5, 10 and 15 minutes, diluted 100-fold in NCDM and plated on the surface of Columbia agar (Grand Island Biologicals) with 4% sheep red blood cells and Isovitalex supplement (Baltimore Biological Laboratories). One-half ml was applied to the surface of each of 4 plates and spread. Plates were incubated at 35°C for 48 hours in an atmosphere of 5% CO_2.

5. Inhibition coefficients were calculated in a similar manner to the Phenol Coefficient. A 10^5-fold reduction in the number of viable cells was the experimental criterion. Those concentrations capable of effecting at least this level of reduction in 10 but not 5 minutes were employed in calculating coefficients.

RESULTS

Comparative disinfection values are presented in Table I. Values obtained with Savlon are minimal values, since at dilutions greater than 1:3200 bacteriostatic effects became virtually impossible to distinguish from bacteriocidal effects. At dilutions of between 1:3500 and 1:4000, a consistent 10^5-fold reduction could not be attained, although reduction was always greater than 10^4-fold. Colonies arising from surviving cells were much smaller than normally experienced with this organism. This phenomenon has also been experienced with artificially aerosolized cells when plated on media containing the antibiotic vancomycin (L.A. White, 1976, Unpublished data) and has been interpreted as an indication of cellular damage.

The cell destruction criterion was consistently attained at dilutions of phenol in the 1:95 to 1:110 range, in the 1:60 to 1:85 range for coal tar and about 1:3200 for Savlon.

DISCUSSION

The results of this study confirm the validity of the decision (A.J. Clayton and J.F. Currie, Unpublished observations, 1973) to use Savlon for the control of *N. meningitidis* in those areas of highest density of surface contamination at CFB Cornwallis; namely, the swimming pool and its associated shower area. Savlon is an extremely effective agent against several strains of this organism. The coal tar disinfectant, on the other hand, at its recommended dilution of 1:80 in water is much less effective.

Meningococcal strains isolated at CFB Cornwallis are highly resistant to death in artificially-generated aerosols at intermediate relative humidity levels (45-50%) (6). Such levels are usual at this base during the December to May period when highest incidence of carrier rate (2) and disease (A.J. Clayton and L.A. White, Unpublished observations, 1973-76) have been observed. In addition, it is conceivable that envelopment in sputum or nasopharyngeal fluid, or association with larger particles (i.e. > 6µm Mass Median Diameter), could enhance survival

of meningococcal cells. It is speculated that both primary aerosol and re-aerosolization of deposited material play a role in the transmission of the carrier state. Therefore, residual disinfectant on surfaces would be advantageous to reduce the secondary aerosol hazard and, from the results obtained, Savlon appears to be the disinfectant of choice in achieving this end. Coal tar disinfectant and phenol are contraindicated in view of their much lower efficacy for destruction of this organism (see Table 1).

The FDA Phenol Coefficient Method and its variations (7) are not suitable for determining the efficacy of Savlon. This agent exerts an apparent bacteriostatic effect on *N. meningitidis* at low concentrations which leads to spurious results. The usual procedure in overcoming bacteriostasis is to add a neutralizer of the agent to the growth tube, or to replace the usual FDA broth with specialized media containing neutralizers, such as Letheen broth (contains lecithin). This medium is the one normally used when quaternary ammonium disinfectants are being tested. No suitable neutralizer exists for Savlon (Ayerst, McKenna and Harrison, Personal communication, 1974) and therefore this approach cannot be employed. In addition, the use of the usual neutralizer for phenol ($FeCl_3$) (9) is not advisable since the concentration of ferric ions has been shown to affect the amount of capsular material in a closely-related species, *N. gonorrhoeae* (8). Ferric ion concentration is also important for the growth of *N. meningitidis* under certain conditions (10).

The technique developed in this study results from an attempt to overcome some of the aforementioned actual or potential problems. To reduce possible complications which might arise due to excess complex organic material, NCDM is used instead of complex media such as Heart Infusion Broth (Difco) or Trypticase Soy Broth (BBL). A 100-fold dilution of the test sample is employed in an attempt to "dilute out" the disinfectant prior to plating and thus reduce or eliminate bacteriostasis. Assay of survival is by plate count because this approach yields more quantitative and reproducible results. This technique is much more demanding in terms of manpower and supplies than those commonly used but is necessary in order to obtain accurate comparisons of the efficacy of these 3 disinfectants on this organism.

CONCLUSIONS

Since the objective for the use of disinfectant in the recruit schools is to reduce the hazard due to secondary aerosol, it is not essential that the agent of choice totally eradicate the organisms but merely that it reduce their numbers to below the hazardous levels. Savlon has been shown to do this quite adequately. Although studies on disinfection of surfaces were not carried out, test tube results were so striking that it is recommended that, 1) Savlon (at a 1:250 dilution in water) be applied daily by spraying in those areas of greatest hazard as determined at CFB Cornwallis (3) (pool and shower) and that, 2) it replace the coal tar-type disinfectant now being used irregularly in scrubbing waters in recruit barracks. These practices should result in reduction of the numbers of *N. meningitidis* on surfaces and possibly decrease the risk of contracting the nasopharyngeal carrier-state or overt disease due to secondary aerosols.

ACKNOWLEDGEMENTS

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TABLE I

EFFECT OF DISINFECTANTS ON *IN VITRO*

SURVIVAL OF *N. MENINGITIDIS* STRAINS

	No. of Tests	S/P[a] Mean	S/P[a] Std. Dev.[b]	C/P[a] Mean	C/P[a] Std. Dev.
2241-C	7	29.7	2.7	0.70	0.12
Slaterus-Y	3	32.3	1.7	0.83	0.06
Air Isolate-B	3	29.6	0.8	0.73	0.12
Cough Isolate-B	3	31.3	2.7	0.77	0.12

[a] S/P = Savlon/Phenol; C/P = Coal tar/Phenol: Ratios of that dilution of disinfectant effecting at least a 10^5-fold reduction in viable cell numbers in 10 min. but not in 5 min. at 35°C.

[b] Standard deviation.

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KEY WORDS

Neisseria

meningococci

meningitidis

disinfection

Savlon

Phenol coefficient

Secondary Aerosol

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